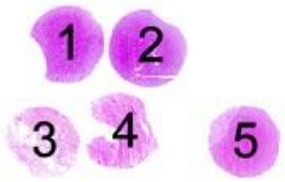
Assessment Run 19 2007

Thyroid transcription factor-1 (TTF1)

The slide to be stained for Thyroid transcription factor-1 (TTF1) comprised:
1. Lung adenocarcinoma, 2. Lung carcinoid, 3. Lung, 4. Thyroid gland, 5. Liver.
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a TTF1 staining as optimal included:

- A strong, distinct nuclear staining of the pneumocytes type II and the Clara cells in the lung.
- A strong, distinct nuclear staining of all the follicular cells and C-cells in the thyroid gland.
- A strong nuclear staining of the majority of the neoplastic cells in the lung adenocarcinoma and at least a weak to moderate, distinct nuclear staining of the majority of the neoplastic cells of the lung carcinoid.

No staining of other cells should be seen except a granular cytoplasmic reaction with mAb clone 8G7G3/1.

99 laboratories submitted stains. At the assessment 15 achieved optimal marks (15 %), 9 good (9 %), 59 borderline (60 %) and 16 (16 %) poor marks.

The following Abs were used:
mAb clone **8G7G3/1** (Dako n=56; NeoMarkers n=11; Ventana n=6; Eurodiagnostica, n=1)
mAb clone **SPT24** (Novocastra, n=25)

Optimal staining for TTF1 in this assessment was only obtained with the mAb clone **SPT24** (15 out of 25 laboratories = 60%).
The protocols giving an optimal result with SPT24 were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (12 out of 16), Cell Conditioning 1 (CC1 Ventana; 1 out of 1), Target Retrieval Solution (TRS Dako pH 9.9; 1 out of 1) or Bond Epitope Retrieval Solution 2 (BERS 2, Vision BioSystem; 1 out of 1). The Ab was used in the range of 1:40 – 1:500 depending on the total sensitivity of the protocol employed. Using these settings 19 out of 20 laboratories (95%) produced a sufficient staining (optimal or good).

With mAb clone **8G7G3/1** only two out of 74 laboratories (3%) were marked as good while the rest obtained borderline or poor marks - primarily due to a false negative reaction of the lung carcinoid but also due to a generally weaker nuclear reaction in cells expected to stain, as well as cytoplasmic cross reaction, not only in liver cells but also in some tumours. The two protocols giving good marks were based on efficient HIER and a highly sensitive detection system. In these two cases the carcinoid showed an unequivocal nuclear reaction, but at the same time a strong cytoplasmic reaction (due to Ab cross reaction). If the lung carcinoid were excluded from the assessment, 59 out 74 (80%) laboratories using the mAb clone 8G7G3/1 achieved a sufficient mark.

The most frequent causes of an insufficient staining were:
- Less successful primary Ab
- Too low concentration of the primary Ab
- Insufficient HIER (Citrate pH 6)

In the assessment the prevalent feature of the insufficient results was a false negative staining of the lung carcinoid with clone 8G7G3/1. Insufficient results with the SPT24 were characterized by a generally too weak reaction of the cells expected to be demonstrated.
Normal lung and thyroid are suitable for control tissue: The nuclear staining should be as strong as possible without significant cytoplasmic reaction. Preferentially, tissue with low antigen content should also be included such as selected cases of well-differentiated pulmonary carcinoid, provided that clone SPT24 is used.

TTF1 was also assessed in run 9, 2003, where 63 laboratories participated out of which 38 (60%) obtained a sufficient mark. In run 9 both the mAb clone 8G7G3/1 and the clone SPT24 could give optimal results (the slide contained a lung carcinoid that also could be stained with clone 8G7G3/1). However, also in that run the pass rate of SPT24 was superior to that of 8G7G3/1 (see table).

	Run 9 2003		Run 19 2007		Total	
	Protocols	Sufficient	Protocols	Sufficient	Protocols analyzed	Sufficient
MAb clone 8G7G3/1	53	28	74	2	127	30 (24%)
MAb clone SPT24	10	10	25	22	35	32 (91%)

Conclusion
Based on the two assessments it appears that the mAb clone SPT24 is the most robust and sensitive marker for the demonstration of TTF1. The mAb clone 8G7G3/1 has a lower sensitivity especially for carcinoid tumours. Please carefully read the TTF1 epitope description as TTF1 occurs in more tumour types than previously described, when using clone SPT24. Efficient HIER is mandatory.

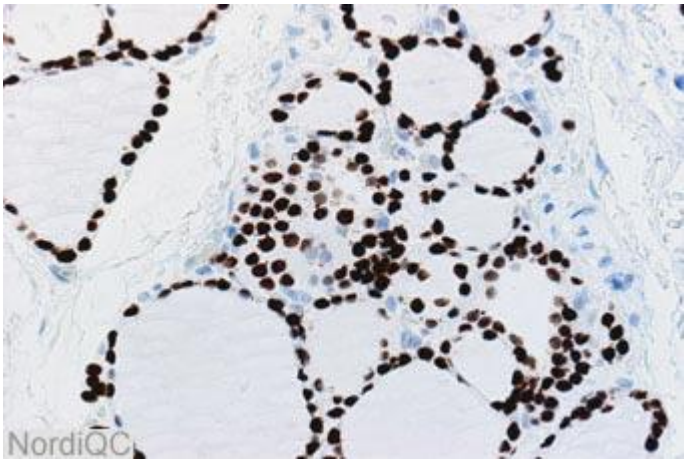


Fig. 1a
Optimal staining for TTF1 of the thyroid gland using the mAb clone SPT24 in a correctly calibrated dilution.
All the thyroid epithelial cells show an intense and distinct nuclear reaction. No cytoplasmic or background reaction is seen.

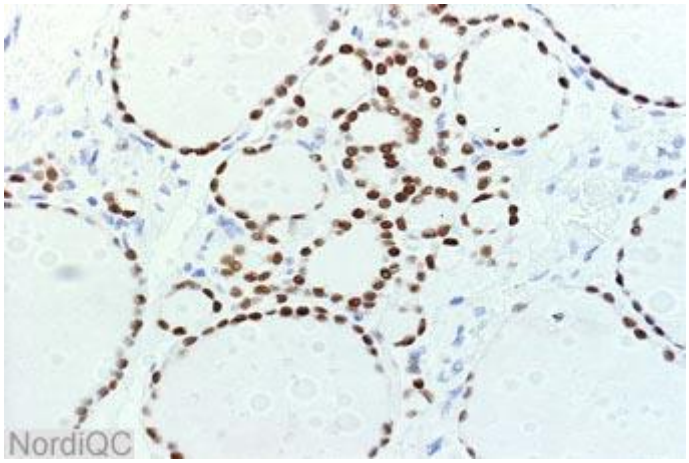


Fig. 1b
Staining for TTF1 of the thyroid gland using an insufficient protocol – mAb clone SPT24 too diluted - compare with Fig. 1a.
The majority of the epithelial cells are demonstrated, but the intensity is only moderate and some cells almost negative.

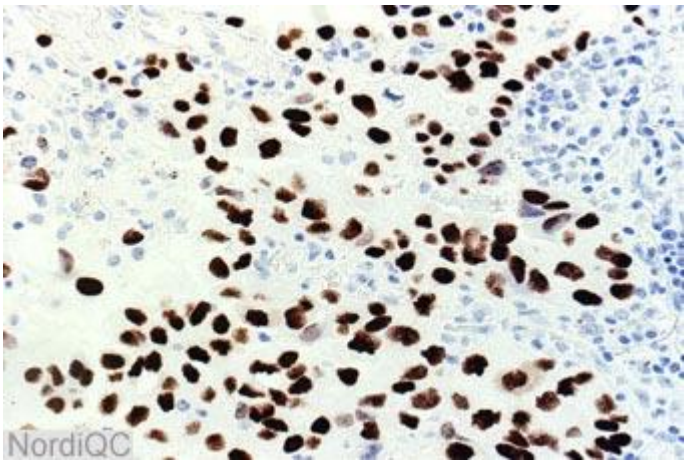


Fig. 2a
Optimal staining with SPT24 for TTF1 in the lung adenocarcinoma. Virtually all the neoplastic cells show an intense and distinct nuclear staining and only a minimal cytoplasmic reaction. Same protocol as in Fig 1a.

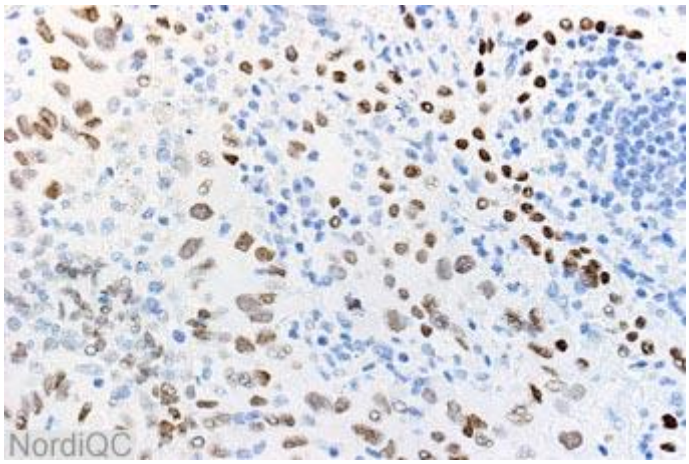


Fig. 2b
Staining with SPT24 for TTF1 in the lung adenocarcinoma using the same insufficient protocol as in fig 1b.
The neoplastic cells are demonstrated, but the intensity is only moderate and some cells almost negative. Also compare with Fig. 3b.

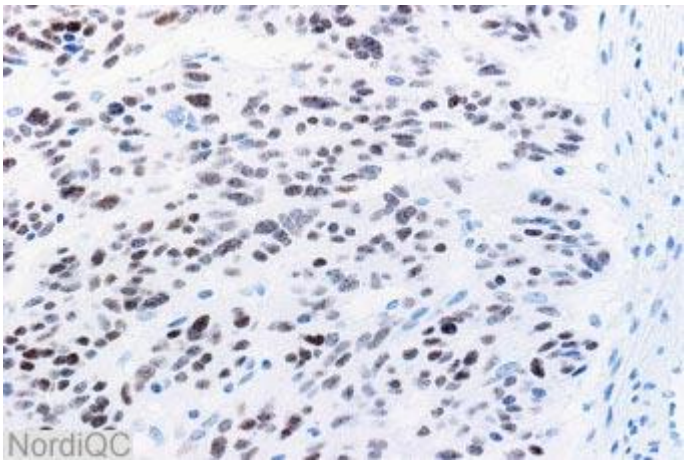


Fig. 3a
Optimal staining with SPT24 for TTF1 of the lung carcinoid. The majority of the neoplastic cells show a moderate, distinct nuclear staining (same protocol as in Figs. 1a and 2a).

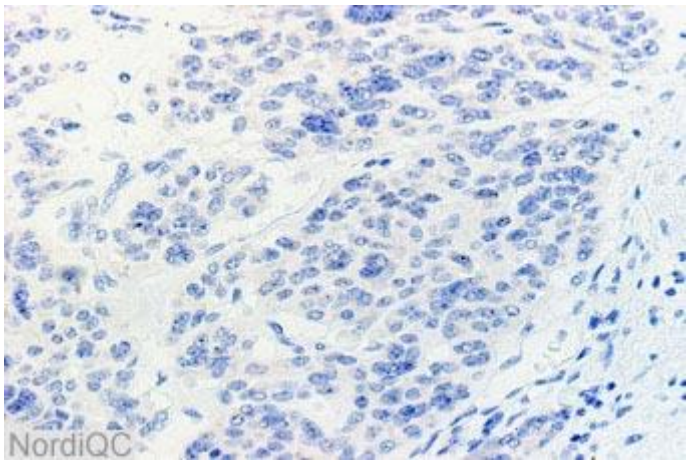


Fig. 3b
Insufficient staining with SPT24 for TTF1 of the lung carcinoid. The neoplastic cells are all false negative (same protocol used in Figs. 1b and 2b).

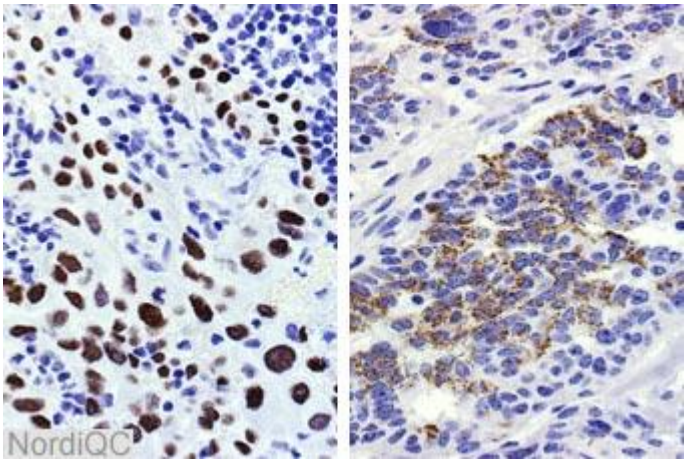


Fig. 4a
Insufficient staining for TTF1 with the mAb clone 8G7G3/1.
Left: The neoplastic cells of the lung adenocarcinoma show an intense nuclear reaction.
Right: The neoplastic cells of the lung carcinoid show a moderate cytoplasmic reaction while the nuclei are negative.
Compare the staining patterns with the optimal stains shown in Figs. 2a and 3a.

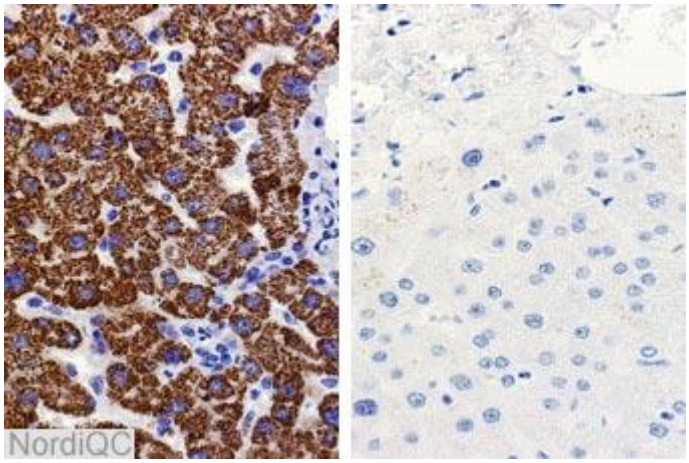


Fig. 4b
Left: Staining of the liver using same protocol as in Fig. 4a based on the mAb clone 8G7G3/1. The hepatocytes show a distinct granular cytoplasmic reaction.
Right: Staining of the liver using same protocol as in Fig. 1a – 3a based on the mAb clone SPT24. The hepatocytes are negative.

SN/MV/LE 2-4-2007